UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4799

16 MAY 2002

APPROVAL SHEET

Title of Thesis: "Application of Headspace Solid Phase Microextraction and Gas Chromatography/Mass Spectrometry for Rapid Detection of the Chemical Warfare Agent Sulfur Mustard"

Name of Candidate: MAJ Gregory L. Kimm
Master of Science in Public Health
Department of Preventive Medicine and Biometrics

Thesis and Abstract Approval:

Chairman: Thomas E. Johnson, PhD	Date
Research Advisor: CDR Philip A. Smith, PhD	Date
COL (ret) Robert J. Fitz, MSPH	Date
COL Robert J. Lipnick, ScD	Date
COL Clifford Cloonan, MD	Date

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
16 MAY 2002	**N/A**	**-**

4. TITLE AND SUBTITLE
Application of Headspace Solid Phase Microextraction and Gas Chromatography/Mass Spectrometry for Rapid Detection of the Chemical Warfare Agent Sulfur Mustard

5a. CONTRACT NUMBER
5b. GRANT NUMBER
5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Kimm /MAJ Gregory L.

5d. PROJECT NUMBER
5e. TASK NUMBER
5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Uniformed Services University of the Health Sciences

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)
11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
A field expedient analytical method for detecting the chemical warfare agent (CWA) sulfur mustard as a soil contaminant was developed using solid phase microextraction (SPME) and gas chromatography/mass spectrometry (GC/MS). Five commercially available SPME fibers were investigated to determine the optimal fiber and extraction conditions. Polyacrylate and carbowax/divinylbenzene fiber coatings gave a statistically indistinguishable and best response compared to the other three types examined in a simple system studied without soil. The polyacrylate fiber coating was selected for study of a system in which sulfur mustard was spiked to an agricultural soil (standard reference material 2709, San Joaquin type). With soil samples, the greatest sensitivity occurred by the addition of deionized water to spiked soil and extraction at ambient temperature for twenty minutes or longer. SPME sampling with GC/MS analyses afforded good reproducibility (relative standard deviation between 2 to 10 %). Analyte concentrations as low as 237 ng g-1 were detected in soil (total ion chromatograms) with this technique. The total time for sampling and analysis was under 1 hour, and the use of organic solvents or special sample introduction equipment was not needed.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**SAR**	**46**	

The author hereby certifies that the use of any copyrighted material in the thesis manuscript entitled:

Application of Headspace Solid Phase Microextraction and Gas Chromatography/Mass Spectrometry for Rapid Detection of the Chemical Warfare Agent Sulfur Mustard

beyond brief excerpts is with the permission of the copyright owner, and will save and hold harmless the Uniformed Services University of the Health Sciences from any damage which may arise from such copyright violations.

Gregory L. Kimm
MAJ, MS, U.S. Army
Department of Preventive Medicine and Biometrics
Uniformed Services University of the Health Sciences

ABSTRACT

Title of Thesis: "Application of Headspace Solid Phase Microextraction and Gas Chromatography/Mass Spectrometry for Rapid Detection of the Chemical Warfare Agent Sulfur Mustard"

Author: MAJ Gregory L. Kimm
Master of Science in Public Health

Thesis Directed by: CDR Philip A. Smith
Assistant Professor
Department of Preventive Medicine and Biometrics

A field expedient analytical method for detecting the chemical warfare agent (CWA) sulfur mustard as a soil contaminant was developed using solid phase microextraction (SPME) and gas chromatography/mass spectrometry (GC/MS). Five commercially available SPME fibers were investigated to determine the optimal fiber and extraction conditions. Polyacrylate and carbowax/divinylbenzene fiber coatings gave a statistically indistinguishable and best response compared to the other three types examined in a simple system studied without soil. The polyacrylate fiber coating was selected for study of a system in which sulfur mustard was spiked to an agricultural soil (standard reference material 2709, San Joaquin type). With soil samples, the greatest sensitivity occurred by the addition of deionized water to spiked soil and extraction at

ambient temperature for twenty minutes or longer. SPME sampling with GC/MS analyses afforded good reproducibility (relative standard deviation between 2 to 10 %). Analyte concentrations as low as 237 ng g^{-1} were detected in soil (total ion chromatograms) with this technique. The total time for sampling and analysis was under 1 hour, and the use of organic solvents or special sample introduction equipment was not needed.

APPLICATION OF HEADSPACE SOLID PHASE MICROEXTRACTION AND GAS CHROMATOGRAPHY/MASS SPECTROMETRY FOR RAPID DETECTION OF THE CHEMICAL WARFARE AGENT SULFUR MUSTARD

BY

MAJ GREGORY L. KIMM

Thesis submitted to the Faculty of the Department of Preventive Medicine and Biometrics Graduate Program of the Uniformed Services University of the Health Sciences in partial fulfillment of the requirement for the Degree of Master of Science in Public Health, 2002

DEDICATION

To my wife, Joan, and my children Michelle and Jacob for the sacrifices you have made during my Army career and the last two years of study. I dedicate this thesis to you.

To my father Gerald Kimm, thank you for the well grounded foundation. It has served me well in life.

In loving memory of my mother, Viola, and brother, Jeff. You two are always with me.

ACKNOWLEDGMENT

I am grateful to the U.S. Army Medical Research Institute for Chemical Defense, especially Dr. Thomas Logan and Mr. Austin Swift, for providing support to complete this work. U.S. Marine Corps Systems Command provided funding to accomplish this research. I am especially grateful to Mr. Adam Becker for providing the funds to the Uniformed Services University.

I would like to thank all the members of my committee for their time and assistance in helping complete this program. I want to thank LTC (Retired) Arthur Lee and COL (Retired) Robert Fitz for designing a program of study relevant to Officers serving as Environmental/Occupational Health professionals. I especially want to thank CDR Phil Smith for all of his mentorship and the analytical capability that he has built. Sir, you truly are Mr. Wizard.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

Figures

Tables

CHAPTER ONE: INTRODUCTION

Statement of the Problem

In both the civilian and military communities, there is growing demand for field analytical detection methods capable of identifying chemical warfare agents (CWA) and toxic industrial materials (TIM). Traditional sampling methods and analytical instrumentation do not easily support rapid sampling, sample preparation or analysis in a field environment. The challenge has been in moving sophisticated analytical instrumentation out of a fixed laboratory environment without compromising instrument sensitivity and collection of reproducible data. Additionally, the demand exists for the creation of sampling and sample preparation methods that reduces both the logistical burden and the handling of hazardous solvents and associated hazardous waste. The primary objective for on-site analysis is to develop a method that combines sampling, sample preparation and analytical detection capable of providing rapid identification of contaminants with a high degree of certainty in order to speed the accomplishment of risk assessment and effective risk communication.

Deployed military personnel may be exposed to a variety of environmental air, water and soil contaminants. Presidential Review Directive 5 established objectives for simple and effective methods to assess troop exposures for environmental pollutants.[1] The strategy for this objective is to develop smaller, lighter, simpler, more sensitive, rugged personal and area environmental samplers and detectors that are capable of measuring and/or sampling multiple exposures/chemicals at toxicologically relevant points.[1]

The application of solid phase microextraction (SPME) and portable gas chromatography/mass spectrometry (GC/MS) offers an effective method for analyzing most CWA and TIM in environmental media such as air, water and soil. GC/MS is an analytical method that has demonstrated field portability and has been used extensively in field settings.[2,3,4,5] The components necessary to perform GC/MS in the field have been miniaturized and made rugged for field use. It is capable of qualitatively identifying a wide range of organic chemical compounds. Development of sampling and sampling preparation methods will increase the sensitivity and the selectivity for detecting and quantitatively measuring organic compounds. SPME is a simplified sampling and sample preparation method that is capable of interfacing with GC/MS.[3,5,] Currently within the Department of Defense, GC/MS capabilities are available at some military units. The U.S. Army's 520th Theater Army Medical Laboratory and the U.S. Marine Corps' Chemical Biological Incident Response Force are capable of supporting deployed forces or providing domestic response support. Presently, neither one of these organizations possesses methods incorporating the use of SPME and GC/MS to analyze contaminants in soil media.

Background

CWA pose a serious and credible threat to civilian and military populations. CWA were used extensively during World War I and 80% of the chemical casualties were caused by sulfur mustard.[6,7] In the more recent past, Iraq used CWA against Iran during the Iran-Iraq war and against Kurdish refugees in the mountainous region of northwest Iraq.[8,9] Soil contaminated with sulfur mustard can remain an exposure hazard

for several years. Black *et al*[8] were able to detect sulfur mustard from soil samples collected almost four years after the employment of a chemical weapon. During the Gulf War, Iraq's possession of CWA was a credible and significant threat to the coalition forces assembled to remove Iraq from Kuwait. Terrorists may use CWA as a weapon of mass destruction. This was demonstrated in 1995 when a religious cult released the CWA Sarin in the Tokyo subway system killing 12 and injuring more than 5,000 people.[6] Additionally, former chemical weapons manufacturing and current demilitarization sites present a potential threat to surrounding areas. There are eight sites within the continental United States and one at Johnston Atoll that fall under the Chemical Stockpile Disposal Program. These stockpile sites, at which leaks and spills have been discovered, pose a potential risk to surrounding communities.[10]

Research Goal

The goal of this research is to develop a rapid sampling and analytical method for the detection of sulfur mustard as a soil contaminant. The rapid identification of chemical contaminants is the first step in an environmental health risk assessment. The Environmental Health Professional requires timely and accurate data in order to assess the hazards associated with chemical compounds of concern. A determination of the risk level associated for the hazard can be calculated and control measures developed to reduce or eliminate adverse health effects. The principles described for solid phase microextraction identified during this research can be readily applied to most CWA and TIM. This methodology will provide timely data on-site and reduce the requirement for sending environmental samples back to a fixed laboratory facility.

Research Questions

Can solid phase microextraction coupled to gas chromatography-mass spectrometry be used to rapidly detect the chemical warfare agent sulfur mustard?

Specific Aims

The specific aims of this research were to: (1) Identify the best SPME fiber for analyzing sulfur mustard, (2) Optimize conditions for extracting sulfur mustard from soil using headspace SPME, (3) Identify degradation products associated with the method, (4) Explore the usefulness of the method on laboratory generated soil samples to measure sulfur mustard

CHAPTER TWO: LITERATURE REVIEW

Sulfur Mustard

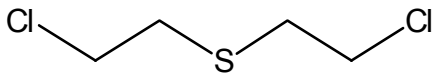
Sulfur mustard (military designation: HD) is classified as a vesicant (or blister) agent. The "H" designates sulfur mustard and the "D" designates a product distilled to a final purity in excess of 92%.[11] The active compound is bis(2-chloroethyl)sulfide. In the liquid or gaseous phase, it will produce blisters to the skin, eyes or lungs due to its strong alkylating properties.[7,11] HD is considered a known human carcinogen.[12] Pertinent physiochemical parameters for HD are listed in Table 1.

Table 1: Sulfur Mustard Physiochemical Properties

Parameter	
Molecular Formula	C_4-H_8-Cl_2-S
CAS	505-60-2
Molecular Weight (g/mole)	159.08
Melting Point (^oC)	13-14
Boiling Point (^oC)	215-217
Vapor Pressure (mm Hg@25º C)	0.11
Vapor Density (Air=1)	5.4
K_{OW}	1.37
K_{OC}	2.12
Water Solubility (g/L)	0.92
Henry's Law Constant (atmxm^3/mole)	2.45 x 10^{-5}
Density/Specific Gravity (g/ml @20º C)	1.247

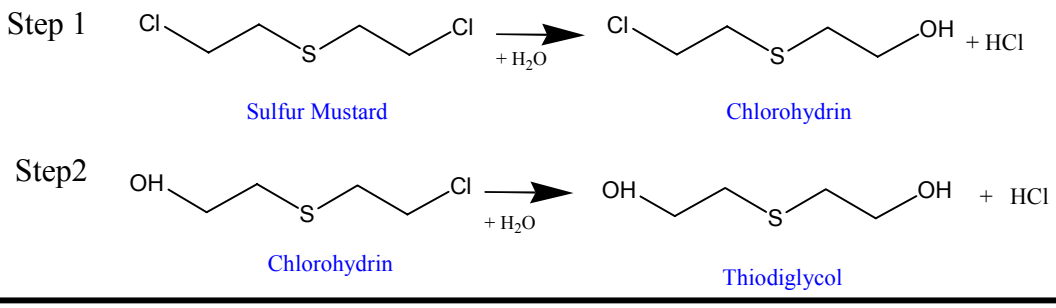
HD is a nonpolar, volatile organic compound possessing two mono-chlorinated ethyl groups bound together around a central sulfur atom. The chlorine atoms have replaced the outer 1º hydrogen along the central axis of the compound.[13] The molecular structure of HD is depicted in Figure 1.

Figure 1. Sulfur Mustard



HD's physiochemical properties and the environmental conditions will determine the environmental fate and transport of this CWA.[11,12] The environmental fate processes include volatilization, hydrolysis and thermal degradation. At temperatures below 14.5º C, HD will solidify and result in little loss due to volatilization. A vapor pressure of 0.11 mm Hg at 25° C indicates that it will exist as a vapor if released into the air. Upon contact with water, HD will undergo a two-step hydrolysis reaction resulting in the production of thiodiglycol (TDG). The hydrolysis reaction is depicted in Figure 2.

Figure 2: HD Hydrolysis



The first step intermediate hydrolysis degradation product is mustard chlorohydrin (CH). The hydrolysis degradation from HD to CH occurs slowly. The solubility of HD is poor when compared to CH; therefore, the first step reaction will primarily occur at the water-HD interface. Substitution of a hydroxyl group for one of the chlorine atoms produces

CH, which is miscible in water. During the second step, CH will hydrolyze at a faster rate than HD. The increased solubility causes the second step hydrolysis reaction of CH to TDG to occur more rapidly.[11,12]

Several factors will affect the volatilization of HD and the production of degradation products. The factors that determine how a chemical compound will interact with soil are sorption, vapor pressure, water solubility and Henry's law constant. A chemical compound's sorption qualities are its ability to form physical and chemical bonds to a soil and it is indicated by its K_{OC} value. The K_{OC} is an adsorption coefficient that expresses the partitioning of an organic compound between a solid phase and a liquid phase. HD's K_{OC} value is 270. A compound with a K_{OC} less than 1000 will not strongly adsorb to soil.[14] Henry's law constant is a proportional expression indicating what concentration of a compound will enter the air, as a partial pressure, relative to the concentration that will remain in water. Roughly, Henry's law constant is the same expression as its vapor pressure divided by its water solubility.[14] This physiochemical property will play a vital role when HD contaminates a moist soil. A Henry law constant value of 2.45×10^{-5} atm $\times$ m^3/mole indicates it will enter the air rather than bond with water molecules. Based upon the K_{OC} and Henry law constant values, HD will volatilize from soil, especially at higher temperatures and conditions of high soil moisture.[15,16] In the absence of soil moisture, HD will undergo thermal degradation and produce by-products due to elimination reactions.[8,9,10] This type of reaction is usually observed at temperatures above 150^0 C; however, the reaction may occur more slowly at ambient temperatures.[11] Thermal/elimination degradation products include 1,2-bis(2-chloroethyl)ethane, 1,4-dithiane and 1,4-thioxane.[8,9,10]

Traditional Extraction Methods

There are several traditional methods for extracting and separating CWA. These methods may afford the ability to detect the target compound at low levels; however, they are not easy to perform nor do they permit rapid analysis in a field setting. Soil is a difficult environmental matrix for detecting CWA contamination. Sample preparation and separation of analytes for soil analysis can require an extensive logistical burden, power requirements, complex analytical instrumentation and dictates the use and handling of hazardous material. The three major methods for preparing a soil sample for analysis involves liquid extraction, Soxhlet extraction or thermal desorption. [8,9,17,18]

Liquid extraction is a process using two immiscible phases to separate a solute from one phase into the other.[19] The analyte will have a greater affinity for the extraction solvent due to chemical properties and becomes mixed in the solvent. The most simplistic liquid extraction method consists of adding an organic solvent to the soil and shaking mechanically or by hand. Ultrasonication is a method of adding a solvent to a solid or soil and using ultrasound to break the adsorptive bond between the analyte and soil.

Soxhlet extraction is another sample preparation technique used to extract organic compounds from solids or soil.[19] The soxhlet extraction apparatus consists of a center chamber containing a porous paper thimble. The thimble is used to contain the soil sample to be extracted. The round bottom flask contains a volatile organic solvent that is heated to create enough solvent vapor to produce a steady flow of liquid drops from the condenser at the top of the soxhlet apparatus. Once the solvent in the upper chamber

rises above the relief arm, the solvent is returned to the round bottom flask and the process repeats itself with the semi-volatile compounds accumulating in the solvent present in the round bottom flask. Most soxhlet extractions take at least four hours and some extractions require twelve to twenty-four hours.

A third method for preparing a sample is by thermal desorption.[8,17] Thermal desorption involves heating a soil sample and causing desorption of the analyte from the soil. One of two methods can be used to trap the desorbed analyte. The first method is to trap the analyte onto a cooled gas chromatography (GC) column. The analyte is volatilized within the GC column upon the termination of cooling by heating the column. The second method for thermal desorption involves trapping the headspace vapor onto a sorbent such as TENAX, XAD or activated carbon. The sorbent is thermally desorbed and the analyte is introduced into a GC.[8] This type of analysis requires the use of sampling pumps and increases the complexity of the method.

Solid Phase Microextraction

Solid-phase Microextraction (SPME) is a solvent free process that combines sampling, extraction, concentration and instrument introduction into a single step eliminating complicated sample preparations methods described previously.[20,21,22] SPME extracts organic compounds onto a thin fused silica fiber coated with a stationary phase material.[20] There are three different extraction modes for SPME: direct, headspace and membrane.[20] In the direct extraction mode, the fiber is placed directly in the water or air sample and the analytes are adsorbed on to or absorbed into the fiber coating directly from the sample matrix. In the headspace mode, a sample of soil or

water is placed into a vial. The SPME fiber is placed in the air directly above the water or soil and analytes partition from the sample matrix through the air to the fiber coating. The air in the vial serves as a barrier between the SPME fiber and the sample matrix. The air barrier protects the SPME fiber and eliminates fouling by high molecular weight compounds and other nonvolatile interferences in the sample media.[20,23] The third SPME extraction mode uses a membrane to protect the SPME fiber from heavily polluted samples, which may cause damage to the fiber.

The fused silica SPME fiber (Figure 3) coated with the sampling phase is housed inside a protective metal sheath when not sampling. The fiber is exposed by extending it beyond the sheath for a given sampling period and then retracting it back into the protective sheath. The protective sheath protects the SPME fiber when piercing a septum on a sampling vial or the GC inlet. Following selective extraction by SPME from air, water or the headspace above water or soil, the analytes are introduced into the analytical instrument with little or no further sample handling.

Figure 3: SPME Sampler

Adjusting the salt concentration, pH and/or extraction temperature optimizes analyte loading onto the SPME fiber. The addition of salt or sample pH control can assist in driving certain compounds from an aqueous sample matrix to the fiber coating. For headspace SPME with solid matrices, increasing the extraction temperature will

assist in dissociating analytes from the sample matrix into the headspace for extraction. At the optimal temperature, net adsorption on to or absorption into the fiber reaches a maximum. At any temperature above the optimal temperature, the analyte will have less affinity for the fiber.[20] The optimal extraction time and temperature for an analyte to reach equilibrium will result in maximum sensitivity. In more complicated samples, higher temperatures than optimal for a simple system can produce relatively more loading where analyte volatilization from the matrix is the limiting factor.

Five types of fibers are commercially available for sampling.[20,21,22,23] Two fiber types have absorptive characteristics. Absorption is a non-competitive process that does not exhaustively extract unless the concentration of the analyte is extremely low and the analyte has a high fiber affinity.[20] During absorption, an analyte dissolves into the SPME coating. The 100 μm Polydimethylsiloxane (PDMS) absorptive fiber possesses an affinity for nonpolar analytes. The other absorptive fiber, 85 μm Polyacrylate (PA), possesses an affinity for polar analytes. The remaining three fibers possess adsorptive qualities. Adsorption is a competitive process where analytes compete for pore space on the SPME coating. The size of the pore space enhances the sensitivity for some analytes based upon molecular size. Adsorptive type fibers use a mixed phase system containing a solid polymer particle, either Divinyl Benzene (DVB) or Carboxen, blended into a liquid phase, either PDMS or carbowax.[20,21,22,23] DVB contains mainly mesopores with some micro- and macropores, which have a tendency to trap analytes ranging from a six to twelve carbon chain compound. Carboxen particles possess an even distribution of micro-, meso- and macropores, which are ideal for analytes ranging from a two to twelve carbon chain compound.[20] Carbowax/DVB has a greater affinity for polar analytes and

PDMS/DVB and Carboxen/PDMS have a greater affinity for bipolar analytes. Carboxen/PDMS is especially useful for sampling small, highly volatile compounds.

Several studies have used SPME headspace analysis for identifying volatile and nonvolatile organic compounds as soil contaminants.[22,24,25,26,27,28,29] Several methods exist for optimizing the extraction efficiencies for specific analytes. As previously described, raising the temperature of the sample will increase the vapor pressure of an analyte causing partitioning of the analyte from the soil matrix into the headspace.[22,24,25] More volatile compounds will partition at lower temperatures and less volatile compounds will partition at higher temperatures. The addition of water to the soil matrix will enhance the recovery of some analytes and improve the sensitivity.[26,27,28] Since adsorption is a surface phenomenon, water will help break the adsorptive bond between the soil and the analyte. For nonpolar analytes, the water will help drive the analyte out of the soil and into the headspace. Analytes capable of hydrogen bonding with the water will not enter the headspace as readily as nonpolar analytes. The Henry's law constant will assist in predicting volatilization of an analyte when water is added to a soil sample.

CHAPTER THREE: METHODS

Materials

HD (97.5% purity) was obtained from the U.S. Army Edgewood Chemical Biological Center (Aberdeen Proving Grounds, MD). HD was handled only after dilution in hexanes. Standards were purchased for 1,4-dithiane (97%), and thiodiglycol (99%) from Aldrich (Milwaukee, WI).

All SPME fibers and holders used in this study were obtained from Supelco (Bellefonte, PA). The following fiber coatings were studied (film thickness as indicated): polydimethylsiloxane (PDMS, 100 μm), polyacrylate (PA, 85 μm), carbowax/divinylbenzene (CW/DVB, 65 μm), polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65 μm) and carboxen/polydimethylsiloxane (CAR/PDMS, 65 μm). Prior to use, each fiber was conditioned following the manufacturer's recommendations. Blank runs were completed at least once daily before use of any fibers for sampling to ensure no carryover of analytes from previous extractions.

Gas Chromatography/Mass Spectrometry Methods

The SPME samples were analyzed immediately following collection using a 6980 series gas chromatograph and 5973 quadrapole mass selective detector (Agilent Technologies, Wilmington, DE). The GC was fitted with a J & W Scientific (Folsom, CA) DB-5, 30 m x 0.25 mm I.D. column having a film thickness of 0.25 μm. Helium at 1 mL/min was used as the carrier gas. The oven was programmed to increase from 35 to 250 °C at 20 °C per minute following a 2.00 min hold time at the initial temperature. Desorption of the SPME fiber samples was accomplished in the splitless injection mode

for 2.00 min, followed by 50 mL/min injector purge. The injector temperature was maintained at 250°C throughout an analysis, and the mass spectrometer transfer line was kept at 270°C. Electron impact ionization (70 eV) was used and mass spectra were collected over the range of 35-350 m/z. Sample retention characteristics and mass spectra were stored using the Agilent Chemstation software package.

Quantitative Analysis of Sulfur Mustard in Solvent

In order to estimate the mass of sulfur mustard loaded onto a SPME fiber, splitless injection analyses of sulfur mustard in solvent were completed by GC/MS to obtain a curve with mass of analyte on-column plotted against total ion current peak area. Three replicate samples were analyzed at each of the five concentrations ranging from 0.7625 to 24.4 ng/mL. The same instrument and conditions as for SPME samples were used, except a split/splitless injection port liner (Agilent) was used in place of the narrow bore liner used for SPME samples. Sample introduction was by autosampler (7673, Agilent) using an injection volume of 1.0 μL.

SPME Sampling

Initial Fiber Selection. SPME fiber selection from among those tested was accomplished in a simple system (no soil) by creating replicate samples from 15 mL vials having open screw top closures fitted with polytetrafluoroethane (PTFE)-lined silicone septa. The vials were spiked with sulfur mustard (2.4 mg/mL in hexanes) by piercing the PTFE-lined silicone septum with a 10 μl syringe (Hamilton, Reno NV) and injecting 5.0 μL of the solution into each vial. To ensure reproducible spiking, a solvent chase method

was used in which 1 μL of hexane was drawn into the syringe, followed by 0.5 μL of air, and then the measured aliquot of sulfur mustard solution. The temperature of the vial sampled was maintained at 25 °C by placing the vial in a digitally controlled hot-block heater (Barnstead/Thermodyne, Dubuque, IA). Each sample was allowed to equilibrate for 10.0 min before the SPME fiber assembly outer sheath pierced the vial septum. Immediately following this, sampling was started when the SPME fiber was lowered through the outer sheath into the headspace of the vial that contained the material to be sampled.

After a 30 min extraction period, the SPME fiber was retracted back into its protective sheath and then removed from the vial and introduced into the heated GC injection port. The sampling fiber was then lowered into the midrange region of the heated injection port liner (0.75 mm I.D. deactivated glass, Supelco) and GC/MS analysis commenced. The fibers giving the highest GC/MS peak areas for the sulfur mustard peak were selected for further sampling and analysis optimization.

Optimization of SPME Extraction Conditions. Another set of spiked vials was analyzed using fibers selected (PA and CW/DVB), under the same set of conditions except the temperature of extraction was increased to 50 or 75 °C to determine the effect of temperature on extraction. Finally, the optimal fibers selected were exposed at the optimal temperature (25 °C) over an increasing extraction time period to determine when equilibrium was reached and no additional net analyte loading occurred with further increase in extraction time.

Experimental data resulting from fiber selection, extraction temperature and uptake curve extraction time analyses were examined for differences between GC/MS

total ion current sulfur mustard peak areas. The statistical test used was the analysis of variance (ANOVA), which was completed for each of the three data sets. As appropriate, this was followed by Tukey's *post hoc* comparison method to evaluate the source of differences observed.

Headspace SPME Soil Extraction. Once the optimal extraction parameters from among those studied had been identified in the simple system, the parameters for extracting sulfur mustard from spiked soil were examined. The soil used was SRM 2709, San Joaquin soil. Soil samples were created by spiking 5.0 μL of 9.5 mg/mL sulfur mustard stock solution onto 1.0 g SRM soil, followed by mixing of the spiked soil within the vial using a vortex mixer for 20 s. The resulting soil concentration was 48 μg sulfur mustard/g soil (48 ppm).

Initial experiments with spiked soil samples were completed with both CW/DVB and PA type fibers. The addition of water to the soil was thought to offer the potential for increased sensitivity. Two sample replicates were collected for both fiber types in soil to which 500 μL deionized water was added 10 min prior to commencement of SPME sampling. Extraction time for these samples was 30 min at room temperature (determined to be 23 ± 0.5 °C). The results were compared using a t-test, and following this work, the CW/DVB fiber was not used further.

Using the PA type fiber, two sample sets were collected. T-tests were performed to compare room temperature and wet soil samples. First, dry soil sample replicates were collected that were created and sampled identically to the PA fiber samples from moist soil, except no water was added. Secondly, experiments were completed with soil prepared identically to the room temperature, wet soil PA samples, except sampling was

completed at 50 and 75 °C. Only the 50 °C replicates from the last sample type mentioned were compared to the room temperature.

To produce a soil system uptake curve, spiked soil sample replicates (n =2, 48 μg in 1.0 g soil) were collected using a PA type fiber at room temperature with water added as per above, at SPME extraction times of 1.0, 5.0, 10.0, 20.0, 30.0, 45.0, and 60.0 min. The resulting data resulting from this extraction uptake curve experiment were examined for differences between GC/MS total ion current sulfur mustard peak areas. An ANOVA, followed by Tukey's *post hoc* comparison method, was performed to evaluate the source of differences observed.

In order to estimate the sensitivity of the method, 30 min extractions were carried out using the PA fiber coating (wet soil) spiked at five concentrations ranging from 95 ng g^{-1} to 475,000 ng g^{-1}. The upper range of these spike samples was determined by chromatography where large, asymmetrical sulfur mustard peaks resulted from the analyte exceeding the capacity of the GC column used. Two replicate samples at each concentration were sampled by SPME at room temperature and at 50 °C, and GC/MS analysis was completed for each sample as for the other samples.

CHAPTER FOUR: RESULTS AND DATA ANALYSIS

SPME sampling with GC/MS analysis gave relative standard deviations (RSD) between 2 to 10 % for all analyses completed using replicate samples. The use of SPME and the optimized extraction parameters specified for this study demonstrates the ability to obtain precise and reproducible results.

Quantitative Analysis of Sulfur Mustard in Solvent

Direct liquid injection analyses of HD in solvent are listed in Table 2. The curve showed good linearity ($r^2 = 0.9826$) as demonstrated by figure 4. By using HD peak area obtained from SPME analyses and solving for mass using the resulting calibration curve, an estimate for the mass of sulfur mustard loaded onto the SPME fiber.

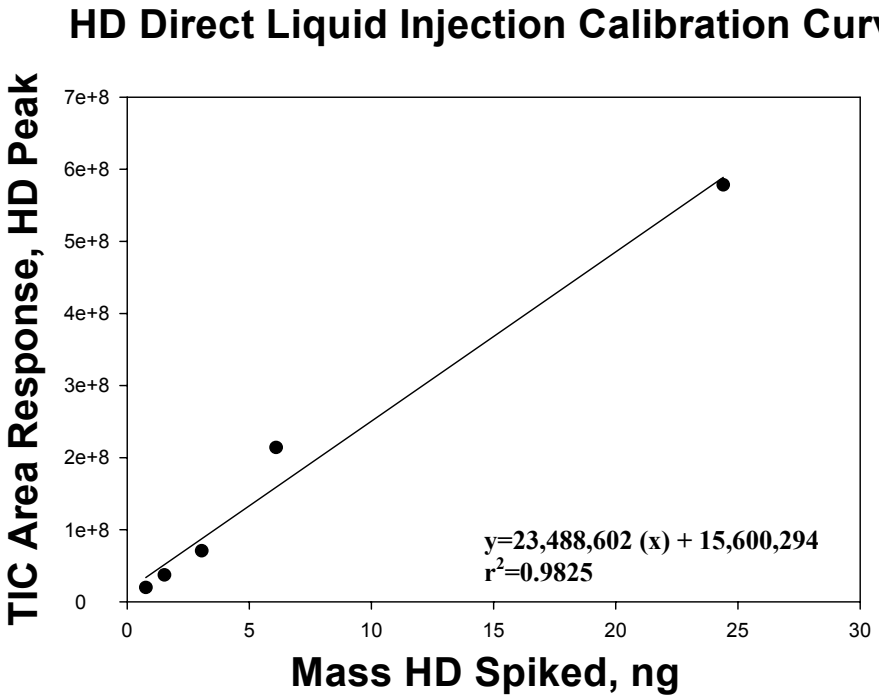
Table 2: Quantitative Analysis of HD:
GC/MS Total Ion Chromatogram[a] Area for HD Peak
Direct Injection onto GC Column

Sample #	0.076 ng	1.525 ng	3.05 ng	6.1 ng	24.4 ng
1	20,027,853	35,507,224	71,817,419	198,523,184	573,132,340
2	18,736,404	37,117,166	69,883,646	219,424,590	581,132,496
3	20,150,035	38,869,221	70,641,157	223,518,306	580,841,676
MEAN	19,638,097	37,164,537	70,780,741	213,822,027	578,368,837
STD DEV	783,275	1,681,499	974,414	13,406,363	4,537,270
RSD[b]	3.99	4.52	1.38	6.27	0.78

a. Table entries are total ion count area response for HD peak

b. Relative Standard Deviation (RSD) = Standard Deviation (STD DEV) / Mean × 100

Figure 4:



SPME Sampling

Initial Fiber Selection. Table 3 shows the data obtained during fiber selection experiments. Figure 5 shows a GC/MS chromatogram for SPME sampling of sulfur mustard from a simple system. The ANOVA test showed that differences existed between GC/MS peak areas that were fiber dependent (Table 3 data, $p<0.001$). A *post hoc* comparison completed on Table 3 data showed that PA and CW/DVB fibers gave an indistinguishable response under the conditions tested ($p = 0.401$), while other fibers differed from each other with significance ($p < 0.001$). The average GC/MS sulfur mustard peak areas for the PA and CW/DVB fibers were greater than for all other fibers tested.

Table 3: Simple System Sampling: SPME Fiber Selection
GC/MS Total Ion Chromatogram Area for HD Peak
30-minute extraction, 25 °C

SPME Fiber Selection

	CW/DVB	PA	PDMS/DVB	100 PDMS	CAR/PDMS
Mean	1,013,351,536	978,731,684	833,541,565	683,167,080	198,375,102
STD DEV	26,111,568	26,791,059	10,504,456	28,653,831	17,484,468
RSD (%)	2.58	2.74	1.26	4.19	8.81

ANOVA $F_{4,5}$ = 621.582
p<0.001

Tukey's *Post Hoc Comparisons* with p values reported

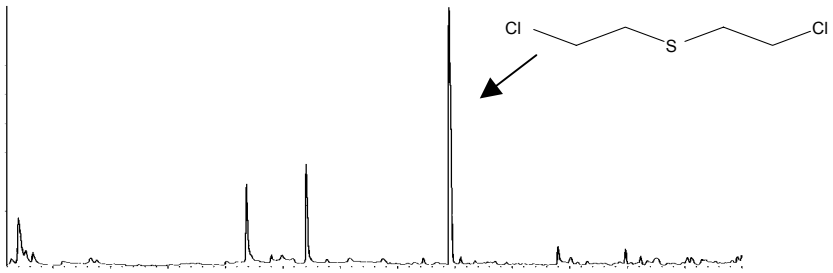
Time	CW/DVB	PA	PDMS/DVB	100 PDMS	CAR/PDMS
CW/DVB	N/A		0.000	0.000	0.000
PA		N/A	0.451	0.000	0.000
PDMS/DVB	0.000	0.451	N/A	0.000	0.000
100 PDMS	0.000	0.000	0.000	N/A	0.000
CAR/PDMS	0.000	0.000	0.000	0.000	N/A

***Post hoc* comparison**

CW/DVB to PA comparison p = 0.401, no significant difference exist between the two fibers

All other fiber comparison p < 0.001, significant difference exist between fibers

Figure 5: GC/MS Chromatogram for HD



Optimization of SPME Extraction Conditions. Table 4 shows the data obtained during temperature selection experiments using the PA and CW/DVB fiber coatings. The ANOVA test completed with these data showed that differences existed between sulfur mustard GC/MS peak areas among the various sample conditions (Table 4 data, $p < 0.001$). A *post hoc* comparison completed on Table 4 data showed that both PA and CW/DVB fibers gave peak areas significantly different from all other combinations of

fiber/temperature tested ($p < 0.001$). As the peak areas for these fibers at 25 °C were greater than all other fiber/temperature combinations, further fiber/temperature combination comparisons are not reported.

Table 4: Simple System Sampling: Optimal SPME Extraction Temperature GC/MS Total Ion Chromatogram Area for HD Peak

CW/DVB Optimal Extraction Temperature, 30 min

	25° C	50° C	75° C
Mean	1,013,351,536	816,573,331	640,729,443
STD DEV	26,111,568	45,228,140	40,221,975
RSD (%)	2.58	5.54	6.28

ANOVA $F_{2,6} = 71.972$
$p<0.001$

Tukey's *Post Hoc Comparisons* with p values reported

Temp	25° C	50° C	75° C
25° C	N/A		0.000
50° C		N/A	0.003
75° C	0.000	0.003	N/A

***Post hoc* comparison**

25° C to 50° C p = 0.002, difference exist between times

PA Optimal Extraction Temperature, 30 min

	25° C	50° C	75° C
Mean	978,731,684	793,456,636	376,370,501
STD DEV	26,791,059	28,157,074	36,257,948
RSD (%)	2.74	3.55	9.63

ANOVA $F_{2,6} = 303.229$
$p<0.001$

Tukey's *Post Hoc Comparisons* with p values reported

Temp	25° C	50° C	75° C
25° C	N/A		0.000
50° C		N/A	0.000
75° C	0.000	0.000	N/A

***Post hoc* comparison**

25° C to 50° C p = 0.001, difference exist between times

The SPME uptake curves completed with the simple system using PA and CW/DVB fibers are presented in Figure 6. Upon observation of the uptake curve for both fibers, an ANOVA test, with Tukey's *post hoc* analysis (Table 5), the significance value ($p = 0.003$) obtained comparing the 20 min uptake samples (CW/DVB) to the corresponding 30 min samples showed that the peak areas were different. The same was shown for the PA fiber 20 min and 30 min samples ($p = 0.006$). Equilibrium was apparently established at 30 min, with possible fugacity losses from the closed system explaining the slight decrease observed at longer extraction times.

Figure 6: HD Uptake curve for CW/DVB and PA Fibers

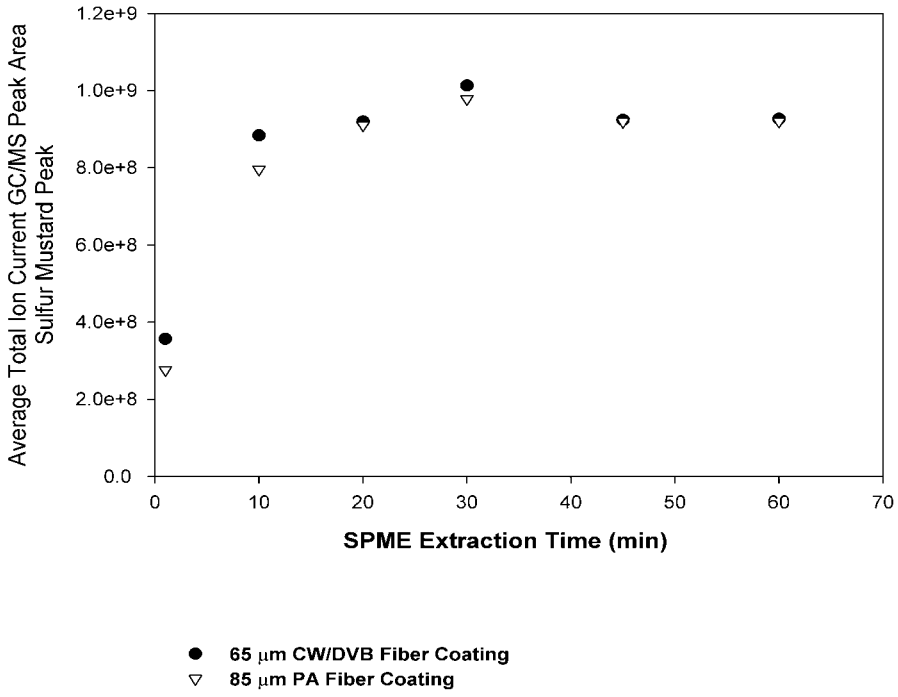


Table 5: Simple System Sampling: Optimal SPME Extraction Time
GC/MS Total Ion Chromatogram Area for HD Peak
25° C Extraction Temperature

HD Upake Curve CW/DVB Fiber, 25 C

	1 min	10 min	20 min	30 min	45 min	60 min
Mean	356,422,186	883,924,943	919,620,553	1,013,351,536	923,771,332	927,060,415
STD DEV	1,510,712	7,105,424	9,738,947	26,111,568	7,673,697	48,137,487
RSD (%)	0.42	0.8	1.06	2.58	0.83	5.19

ANOVA $F_{5,6}$ = 322.006
p<0.001

Tukey's *Post Hoc Comparisons* with p values reported

Time	1 min	10 min	20 min	30 min	45 min	60 min
1 min	N/A	0.000	0.000	0.000	0.000	0.000
10 min	0.000	N/A		0.000	0.343	0.271
20 min	0.000		N/A		1.000	0.998
30 min	0.000	0.000		N/A	0.005	0.007
45 min	0.000	0.343	1.000	0.005	N/A	1.000
60 min	0.000	0.271	0.998	0.007	1.000	N/A

***Post hoc* comparison**

10 to 20 minute comparison p = 0.451, no significant difference exist between times

20 to 30 minute comparison p = 0.003, significant difference exist between times

HD Uptake Curve PA Fiber, 25 C

	1 min	10 min	20 min	30 min	45 min	60 min
Mean	275,838,423	795,974,607	910,766,586	978,731,684	918,877,638	920,308,925
STD DEV	13,617,018	7,504,034	19,892,051	26,791,059	22,859,185	5,248,110
RSD (%)	4.94	0.94	2.18	2.74	2.49	0.57

ANOVA $F_{5,6}$ = 656.754
p<0.001

Tukey's *Post Hoc Comparisons* with p values reported

Time	1 min	10 min	20 min	30 min	45 min	60 min
1 min	N/A	0.000	0.000	0.000	0.000	0.000
10 min	0.000	0.000	0.000	0.000	0.000	0.000
20 min	0.000	0.000	N/A		0.992	0.984
30 min	0.000	0.000		N/A	0.014	0.017
45 min	0.000	0.000	0.992	0.014	N/A	1.000
60 min	0.000	0.000	0.984	0.017	1.000	N/A

***Post hoc* comparison**

10 to 20 minute comparison p < 0.001, significant difference exist between times

20 to 30 minute comparison p = 0.006, significant difference exist between times

Headspace SPME Soil Extraction. Table 6 shows the initial comparisons between CW/DVB and PA fiber coatings for sampling spiked soil (wet soil, 25 °C). The average peak area given with CW/DVB sampling was only 48% that of the samples collected with the PA fiber, a significant difference (2-tailed t-test, $p = 0.023$). It is possible that water interferes with HD's adsorption on to the polar CW/DVB coating, decreasing the sensitivity obtained with this coating (relative to the PA fiber coating) compared to the simple system where water was not added. Table 7 shows the average sulfur mustard GC/MS peak area for the dry, room temperature samples was <1% that of the samples collected at room temperature with wet soil, a significant difference (2-tailed t-test, $p = 0.0024$). The average sulfur mustard GC/MS peak area for the 50 °C, wet soil SPME samples was 8.2% that of the wet soil samples collected at room temperature, a significant difference (2-tailed t-test, $p= 0.003$). In wet soil PA samples, there were no sulfur mustard GC/MS peaks detected in the 75 °C samples.

Table 6: Soil System Headspace SPME:
Optimal SPME Fiber, Water Added to Soil
GC/MS Total Ion Chromatogram Area for HD Peak
30-minute extraction, 25 ºC.

Sample #	CW/DVB	PA
1	1,647,631,951	2,899,789,567
2	1,261,715,232	3,197,400,797
Mean	1,454,673,592	3,048,595,182
STD DEV	272,884,329	210,442,919
RSD	18.76	6.90

RSD = STD DEV / Mean
2 tailed t-test p = 0.023

Table 7: Soil System Headspace PA SPME Fiber:
Optimal extraction parameter: with or without water added to soil,
GC/MS area counts for sulfur mustard
30-minute extraction, 25 ºC.

Sample #	No water	With Water
1	26,063,438	2,899,789,567
2	25,085,231	3,197,400,797
Mean	25,574,335	3,048,595,182
STD DEV	691,697	210,442,919
RSD	2.70	6.90

RSD = STD DEV / Mean
2 tailed t-test p = 0.003

Table 8 shows the data obtained from the SPME uptake curves for the soil system completed with PA fiber. Figure 7 shows the soil system uptake curve. Following the ANOVA test, Tukey's *post hoc* comparisons indicated that equilibrium was established at 20 min, as GC/MS peak areas were not shown to be statistically different when comparing the 20 min sample peak areas with those from extractions carried out for longer periods of time. The peak areas from 10 min samples were significantly different ($p = 0.038$) from those of the 20 min and longer samples. The peak areas from the 20 min samples were not significantly different from the 30 min samples ($p=0.720$); therefore, indicating equilibrium was established in the soil system.

Table 8: Soil System Headspace SPME uptake: Optimal SPME extraction time, GC/MS Total Ion Chromatogram Area for HD Peak 30-minute extraction, 25 ºC.

HD Soil Uptake Curve PA Fiber, 25 C

	1 min	5 min	10 min	20 min	30 min	45 min	60 min
Mean	308,059,035	1,117,983,464	1,730,768,469	3,061,265,377	3,548,589,890	3,221,616,196	3,082,037,779
STD DEV	16,143,106	61,987,778	562,543,643	339,810,038	220,708,116	56260581	463,655,208
RSD (%)	5.24	5.54	32.50	11.1	6.22	1.75	15.04

ANOVA $F_{6,7}$ = 30.834

p<0.001

Tukey's *Post Hoc Comparisons* with p values reported

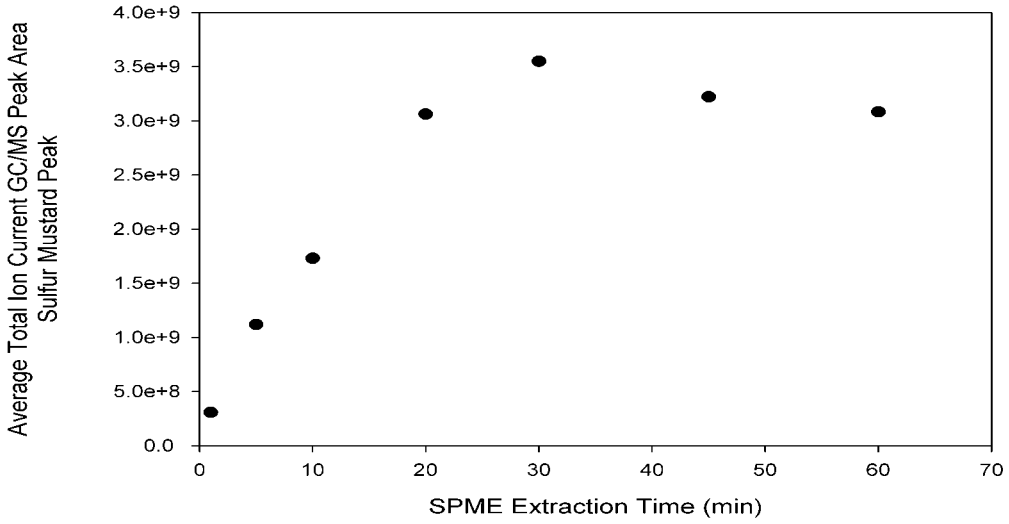
Time	1 min	5 min	10 min	20 min	30 min	45 min	60 min
1 min	N/A	0.266	0.027	0.001	0.000	0.000	0.001
5 min	0.266	N/A	0.517	0.005	0.001	0.003	0.005
10 min	0.027	0.517	N/A	0.038	0.007	0.022	0.035
20 min	0.001	0.005	0.038	N/A	0.720	0.998	1.000
30 min	0.000	0.001	0.007	0.720	N/A	0.930	0.753
45 min	0.000	0.003	0.022	0.998	0.930	N/A	0.999
60 min	0.001	0.005	0.035	1.000	0.753	0.999	N/A

***Post hoc* comparison**

10 to 20 minute comparison p = 0.038, significant difference exist between times

20 to 30 minute comparison p = 0.720, no significant difference exist between times

Figure 7: HD Uptake curve with PA Fiber



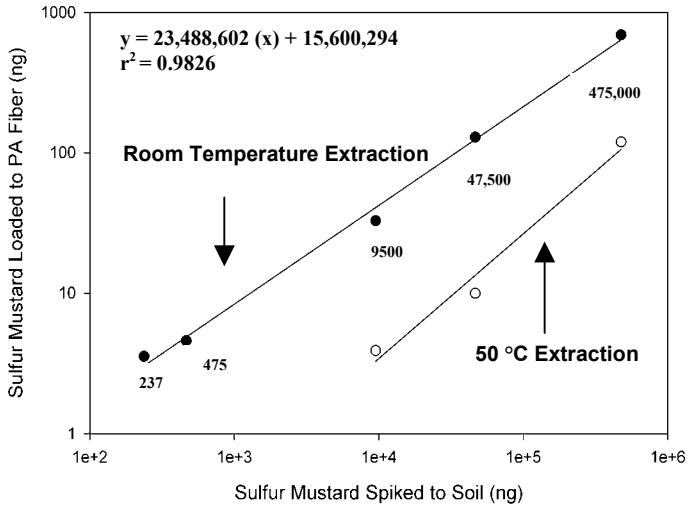
Analysis of the SPME samples ranging from 95 to 475,000 ng sulfur mustard spiked per g soil (wet soil, room temperature extraction) listed Table 9 gave good linearity (r^2=0.9925) as demonstrated in Figure 8. In examining total ion and extracted ion (109 *m/z*) traces for the sulfur mustard peak, it was not observed in samples spiked at 95 ng g^{-1} concentrations. At room temperature, sulfur mustard peaks were observed at a signal-to-noise ratio greater than 3:1 at 237 ng g^{-1}. At 50 °C, no peaks were observed at concentrations below 9,500 ng g^{-1}.

Table 9: Soil limits of detection for Sulfur Mustard on San Joaquin soil

Sample #	237 ng	475 ng	9.5 ug	48 ug	475 ug
1	100,468,785	114,940,339	754,807,890	2,899,789,567	16,884,194,578
2	97,455,699	131,475,800	818,283,976	3,197,400,797	14,725,754,823
Mean	98,962,242	123,208,070	786,545,933	3,048,595,182	15,804,974,701
STD DEV	2,130,574	11,692,337	44,884,371	210,442,919	1,526,247,388
RSD	2.15	9.49	5.71	6.90	9.66

Relative Standard Deviation (RSD) = Standard Deviation (STD DEV) / Mean × 100

Figure 8: Detection of HD from soil with PA Fiber



Although maximum sensitivity was the desired endpoint in the work performed, the use of room temperature extraction for as little as 10 min from a soil system similar to the SRM soil used would give about half the sensitivity of the 20 min or longer extraction time as shown in Figure 7. Combined with the 10 min equilibration time that we used following addition of water before starting sampling, and an analysis time of about 15 min by the GC/MS method used, a single sample could be completed in as little as 35 min with the methods described here. The effect of shortening equilibration time was not tested and the full 10 min time may not be needed, giving yet even shorter sampling and analysis time.

Several degradation products were identified in soil sample systems by SPME sampling and GC/MS analysis. Retention time and mass spectrum matches were obtained for 1,4-dithiane and thiodiglycol. The thiodiglycol was observed only at 75 °C sampling temperatures, and the resulting peaks were small. Another compound was detected by GC/MS and tentatively identified as bis(2-chloroethyl)disulfide by mass spectrum library search and match only, as no chemical standard was available for this compound. 1,4-dithiane and bis(2-chloroethyl)disulfide, were identified in all soil systems to which water was added (ambient temperature, 50 and 75 °C). These analytes are known degradation products of sulfur mustard.[12]

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

Conclusion

HD was sampled by SPME in simple systems, and as a contaminant of SRM agricultural soil, with analysis by GC/MS. On examination of commercially available SPME fiber coatings and different extraction conditions using a system without soil, PA and CW/DVB fiber coatings were shown to be similar and gave larger sulfur mustard GC/MS peak areas compared to the other fibers tested. Other researchers have demonstrated the usefulness of wetting soil samples for headspace SPME sampling/analysis for analytes that are not miscible in water.[27,28,29] For headspace SPME sampling with contaminated soil, the addition of water to the spiked soil increased partitioning of sulfur mustard to the headspace, and with sampling times of 20 min or longer at ambient temperature gave the best sensitivity. Under these conditions, the PA fiber coating was deemed a better choice compared to the CW/DVB coating. SPME sampling with GC/MS analyses afforded good reproducibility (RSD between 2 to 10 %), and analyte concentrations as low as 237 ng g^{-1} were detected in soil (total ion chromatograms).

As demonstrated by this study, SPME combined with GC/MS is a sampling, sample preparation and analytical method well suited for field analysis. The total time for sampling and analysis was just under 1 hour, and use of solvents or special sample introduction equipment was avoided. This reduces the logistical burden and the footprint required to perform this type of analysis in a field environment. Black *et al.*[9] successfully detected sulfur mustard from soil samples using active headspace sampling and full scan GC/MS.[8] Their sampling and analyses were completed rapidly (about 30 min) for sulfur

mustard in soil, by pumping soil headspace air through a tube loaded with TENAX for thermal desorption and GC/MS analysis. The thermal desorption apparatus is a large piece of equipment and adds complexity to the analysis. Additionally, they used solvent extraction of soil with more traditional laboratory procedures to process the soil samples examined in their work. This created the requirement for hazardous solvents and supporting laboratory glassware; therefore, these methods are not as suitable for field analysis as the SPME methods.

Recommendation

Further research is warranted on developing methods for the rapid analysis of other CWA and common TIM in a field environment. Currently within the Department of Defense, there are several organizations possessing GC/MS analytical instrumentation. These organizations support both deployed military forces during contingency operations and provide domestic support in the event of a weapon of mass destruction employment or some other type of catastrophic release of TIM within the United States. These organizations do not have the capability or resources to perform research in developing rapid analytical methods for the detection and identification of CWA and TIM. The organizations responsible for identification of chemical compounds of public health concern must receive appropriate training and experience on the use of methodologies and analytical instrumentation. Specifically, training must address the capabilities and the limitations associated with sampling strategies and analytical methods. Foremost, there must be an understanding that no one "black magic" box can perform any analysis by an untrained/under-trained technician. Additionally, organizations capable of

performing field identification of CWA or TIM should undergo a validation process in order to insure that they are able to meet an established standard for analytical identification.

Limitations of the Study

One limitation concerning this study was the evaluation of only one soil type. Soil will vary from one area to the next, as it is a complex mixture of organic and inorganic substances derived from local materials. The organic carbon content is an important soil characteristic for predicting adsorption of chemical compounds. The inorganic components, such as silica, metals and clay, will also potentially influence chemical soil adsorption. The soil used in this study, a Standard Reference Material (SRM) 2709, San Joaquin soil (National Institute of Standards and Technology, Gaithersburg MD), is an agricultural soil with a high organic carbon content; therefore, it represents one of the more difficult soils for performing SPME headspace analysis. The soil limits of detection measurements established with the San Joaquin soil cannot be applied directly to other soils; however, the study will give a limit of detection for soil characterized as one containing a high amount of organic carbon. A reasonable generalization could be made that a soil possessing low organic carbon content would adsorb less sulfur mustard resulting in a higher concentration of analyte measured by a SPME headspace method.

Sulfur mustard degradation products found in the environment may be different than those identified in this study. The degradation products found in the environment would serve only as qualitative indicators of sulfur mustard contamination. The laboratory generated soil samples will not have the same set of dynamic conditions as soil

in the environment. Other factors such as microbial degradation, temperature, moisture gradients and varying soil composition were not evaluated in this study.

LITERATURE CITED

1. National Science and Technology Council, "A National Obligation: Planning for Health Preparedness for and Readjustment of the Military, Veterans, and Their Families after Future Deployments". August 1998

2. Baykut G and J Franzen. Mobile mass spectrometry; a decade of field applications. *Trends in Analytical Chemistry*. 1994; 13: 267-275

3. Eckenrode BA. The application of an integrated multifunctional field-portal GC/MS system. *Field Analytical Chemistry and Technology.* 1998; 2(1): 3-20

4. Eckenrode BA. Environmental and forensic applications of field-portable GC/MS: an overview. *Journal of the American Society forMass Spectrometry.* 2001; 12: 683-693

5. Smith PA, TA Kluchinsky, PB Savage, RP Erickson, AP Lee, K Williams, M Stevens and RJ Thomas. Traditional sampling with laboratory analysis and solid phase microextraction sampling with field gas chromatography/mass spectrometry by military industrial hygienist. *Journal of the American Industrial Hygiene Association.* 2002; 63: In press

6. Kientz CE, Chromatography and mass spectroscopy of chemical warfare agents, toxins and related compounds: state of the art and future prospects. *Journal of Chromatography A*. 1998; 814: 153-193

7. Somani, SM and JA Romano, Ed. Chemical Warfare Agents: Toxicity at Low Levels. (Washington, D.C.: CRC Press, 2001) 447

8. Black RM, RJ Clarke, DB Cooper, RW Read and D Utley. Application of headspace analysis, solvent extraction, thermal desorption and gas chromatography-mass spectroscopy to the analysis of chemical warfare samples containing sulfur mustard and related compounds. *Journal of Chromatography* 1993; 637: 71-80

9. Black RM, RJ Clarke, RW Read and MTJ Reid. Application of gas chromatography-mass spectroscopy-tandem mass spectroscopy to the analysis of chemical warfare samples, found to contain residue of the nerve agent sarin, sulfur mustard and their degradation products. *Journal of Chromatography A*. 1994, 662: 301-321

10. National Research Council. Recommendations for the disposal of chemical agents and munitions. Washington, D.C.: National Academy Press; 1994. pp 34-51

11. Small MJ. Compounds formed from the Chemical Decontamination of HD, GB and VX and their environmental fate. Technical report 8304: AD A149515. Fort Detrick, MD: U.S. Army Medical Bioengineering Research and Development Laboratory, 1984

12. Munro NB, SS Talmage, GD Griffin, LC Waters, AP Watson, JF King and V Hauschild. The sources, fate and toxicity of chemical warfare degradation products. *Environmental Health Perspectives* 1999; 107(12): 933-973

13. Compton, JAF. Military and Chemical Properties: Chemical and Toxicological Properties. (Caldwell, NJ, The Telford Press, 1987) 458

14. Connell, DW. Basic Concepts of Environmental Chemistry. (New York, NY, Lewis Publishers, 1997) 506.

15. Hazardous Substance Database. Bis(2-chloroethyl)sulfide. Bethesda, MD. http://www.sis.nlm.nih.gov

16. Ney, RE. Fate and Transport of Organic Chemicals in the Environment: A Practical Guide. (Rockville, MD, Government Institutes, Inc., 1998) 370

17. Witkiewicz, Z., M. Mazurek and J. Szulc. Chromatographic analysis of Chemical Warfare Agents. *Journal of Chromatography* 1990; 503:293-357

18. D'Agostino PA, LR Provost. Determination of Chemical Warfare Agents, Their Degradation Products and Related Compounds. *Journal of Chromatography* 1992; 589: 287-294

19. Instrumental Chemistry. Soxhlet extraction. http://www.istrumentalchemistry.com/sampleprep/pages/soxhlet.htm.

20. Pawliszyn, J, ed. Applications of Solid Phase Microextraction. (Hertfordshire, U.K. Royal Society of Chemistry, 1999) 655

21. Müller, L.; Górecki, T.; Pawliszyn, J. Optimization of the SPME device design for field applications. *Fresenius Journal of Analytical Chemistry.* 1999, 364, 610-616

22. Zhang, Z.; Yang, M.J.; Pawliszyn, Headspace Solid-Phase Microextraction. *Journal of Analytical Chemistry*. 1994, 66: 844A-853A

23. de Fatima Alpendurada M. Solid Phase Microextraction: A promising Technique for Sample Preparation in Environmental Analysis. *Journal of Chromatography A*. 2000, 889: 3-14

24. Havenga WJ and ER Rohwer. Chemical Characterization and Screening of Hydrocarbon Pollution in Industrial Soils by Headspace Solid-Phase Microextraction. *Journal of Chromatography A*. 1999, 848: 279-295

25. Eriksson M, J Faldt, G Dalhammar, AK Borg-Karlson. Determination of hydrocarbons in old cresote contaminated soil using headspace solid phase microextraction and GC-MS. *Chemosphere*. 2001, 44: 1641-1648

26. Ng WF, Teo MJK, HA Lakso. Determination of organophosphorous pesticides in soil by headspace solid phase microextraction. *Fresenius Journal of Analytical Chemistry*. 1999, 363: 673-679

27. Fromberg A, T Nilsson, BR Larsen, L Montanarella, S Facchetti, JO Madsen. Analysis of chloro- and nitroanilines and benzene in soils by headspace solid-phase microextraction. *Journal of Chromatography A*. 1996, 746: 71-81

28. Santos FJ, MN Sarrion, MT Galceran. Analysis of chlorobenzenes in soils by headspace solid-phase microextraction and gas chromatography-ion trap mass spectrometry. *Journal of Chromatography A*. 1997, 771: 181-189

29. Sarrion MN, FJ Santos, MT Galceran. Strategies for the analysis of chlorobenzenes in soils using solid-phase microextraction coupled with gas chromatography- ion trap mass spectrometry. *Journal of Chromatography A*. 1998, 819: 197-209